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ORIGINAL ARTICLE

Synthesis and conjugation of ZnO nanoparticles with bovine serum albumin for biological applications

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Abstract Semiconductor nanomaterials tagged with biomarkers may be used for an early fluorescence-based detection of breast cancer. ZnO nanoparticles are water-soluble, non-toxic, photo-chemically stable with highly fluorescence applicability and are regarded for their possible biocompatibility. As a long-term research planning, we are aiming to use QDs conjugated with serum-biomarker for the diagnosis of breast cancer. The present work is a part in the said direction and reports preliminary observations on the synthesis and conjugation of ZnO nanoparticles with a representative protein marker.

Keywords ZnO nanomaterials · Quantum dots · Breast cancer · Biomarkers

Introduction

Quantum dots (e.g., CdS, CdSe, CdTe, ZnS, PbS) are one of the nanoparticles that are used in imaging, detection and targeting. These are nanometre-size luminescent semiconductor crystals and have unique chemical and physical properties due to their size and their highly compact structure (Nie et al. 2007; Ferrari 2005; Langer 1998; Duncan 2003; Li et al. 2004; Allen 2002). They emit different wavelengths over a broad range of the light spectrum from visible to infrared, depending on their size and chemical composition (Moussodia et al. 2008). Eventual use of nanoparticles to dramatically improve clinical diagnostic tests for the early detection of cancer and other (Meulenkamp 1998; Jain 1998). The use of quantum dots heralds a revolution in biological imaging. The current and widely used organic fluorophores have two shortcomings associated with their fluorescence (Srinivas et al. 2002). Signals from the labelled molecules can be obscured by cell auto fluorescence, occurring in the visible spectrum and by photobleaching, which seriously limits observation time (Neuwalt et al. 2004; Gondal et al. 2009). Colloidal quantum dots are bright, photostable fluorophores of a few nanometers in diameter. Because their size approximates that of individual biomolecule, water-soluble nanoparticles complex have been used to target and image tumour cells. Despite their advantages, the best materials for nanoparticles cadmium sulphide, CdS and cadmium selenide, CdSe can be highly toxic because of quantum confinement region span the entire optical spectrum. Cd ions are able to bind to thiol molecules in mitochondria to cause significant cell death (Gorla et al. 1999). These NPs may also damage DNA and disrupt cell activity from factors such as surface coating them where we prepare biocompatible semiconductor NPs like ZnO remarkable photochemical stability and several properties, including size-dependent broad emission, very high extinction coefficient, readily size-tunable narrow emission, high fluorescence quantum yields and use for disease diagnostics like cancer, neurological disorders etc. (Haase et al. 1988; He et al. 2008; Singh et al. 2005; Thareja and Shukia 2007; Wang et al. 2003; Wong et al. 1998). Proteins conjugation like BSA with nanoparticles attempts have been made for applications such as fluorescence labelling, immunoassays including FRET, disease diagnosis etc. (Mohanta et al. 2008; Xie et al. 2003; Terpetsching et al. 1994). The presence of antibodies that interact with BSA might serve as a diagnostic tool for detection of high-risk patients Kagn Kerman

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(2007). The presence of antibodies reacting with BSA in sera from HEV-infected patients may play a major pathogenetic role by the generation of autoantibodies.

Presented work includes an overview of applications of fluorescence microscopy for the diagnosis of cancer with special emphasis on ZnO nanoparticles. As an alternative approach for the synthesis of the nanoparticles, chemical route has been suggested for the synthesis of ZnO NPs, which have again been conjugated with BSA. The above steps may be useful for developing an immunosensing method based upon the use of NPs.

Experimental details

Materials and general method

All the solvent and reagents were analytical grade, commercially available and used as received. The UV–VIS spectroscopy (Varian, carry 500) studies were carried out in range 200–3,300 nm. The topological studies were carried out using field emission scanning electrons microscope-energy dispersive X-ray spectroscope (FESEM-EDX Hitachi S4800 SE), dynamic light scattering (DLS) (Brookhaven, BI-200SM). The functionalization and sensing was taken using photoluminescence spectrophometer (Varian, carry 500).

Synthesis of ZnO nanoparticles

The ZnO nanoparticles were synthesized according to *Ralph-olivier Moussodia* method. Zinc acetate (220 mg) was dissolved in hot ethanol (20 ml) under vigorous stirring. Oleic acid (70 µl) was then added and the mixture was refluxed. In a separate flask, tetramethylammonium hydroxide (360 mg) was dissolved in refluxing ethanol (5 ml). This solution was then rapidly injected in the solution containing zinc acetate and oleic acid. The whole contents were refluxed for 2 min, and then diluted with EtOH (50 ml) followed by cooling to 0 °C. A white precipitate of ZnO nanoparticles appeared. The particles were centrifuged (15 min at 4,000 rpm) for the removal of supernatant. The resulting oleate capped ZnO QDs were washed several times with ethanol. After washing, the ZnO nanoparticles were suspended in 10 ml of toluene and stored in dark at 4 °C.

Conjugation of bovine serum albumin

Before conjugating ZnO nanoparticles with BSA, 5 ml of ZnO suspension was mixed with 5 ml of 5.0 mg/ml NHS (made in 100 µl PBS). After 10 min of reaction, the pH of the solution was adjusted to 7.4 by borate buffer. The conjugation of ZnO nanoparticles with BSA was achieved by incubating the above solution with 1 ml of 1.0 mg/ml BSA for 90 min at 37 °C. The contents were stored at 4 °C.

Results and discussion

Surface morphological studies have been carried out for determining the surface texture of the ZnO nanoparticles. The results of these studies are explained in the following section:

FESEM image of ZnO nanoparticles showing their morphological parameters and EDX pattern suggests the confirmation for synthesis of ZnO nanoparticles. From both the synthesis routes, the ZnO nanoparticles of <5 nm could be synthesized. However, the agglomeration of these nanoparticles was observed in the case where the procedure 2 was adopted. DLS revealed unimodal population corresponding to dispersion of ZnO nanoparticles in Fig. 2. DLS studies indicates that a D_h of 15–50 nm nanoparticles size range due to some degree of agglomeration.

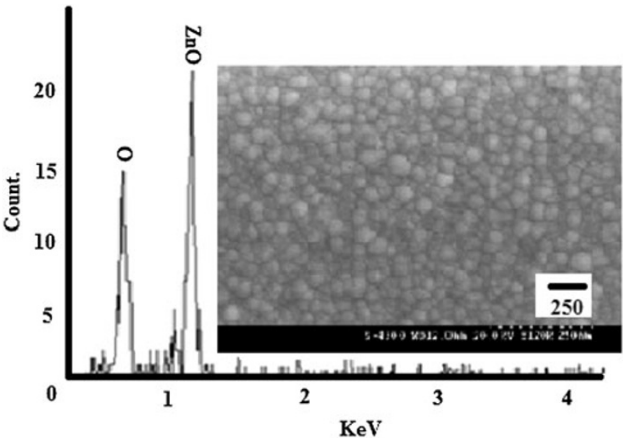

Fig. 1 FESEM image of with EDX for ZnO nanoparticles (<5 nm)

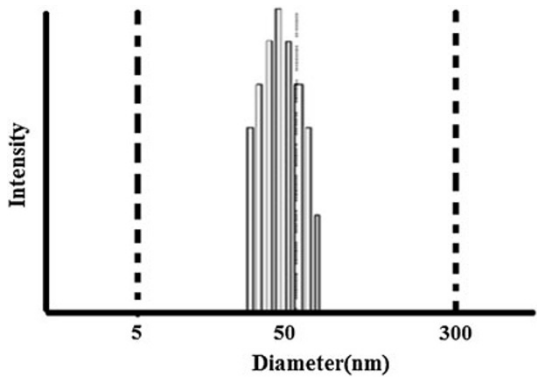

Fig. 2 DLS graph showing particles size distribution of ZnO nanoparticles

For long-term storage, it was found more useful to synthesize the ZnO nanoparticles by following the first suggested procedure. UV–Vis spectra of such synthesized nanoparticles are given in Fig. 3. Upon comparison with the reported literature data, it is evident that the prepared nanoparticles have typical characteristics of ZnO crystals and their diameter, calculated by theoretical simulations should lie below 3–4 nm.

A UV–Vis spectrum of these ZnO nanoparticles, shown in Fig. 2, gives broad absorption band. Such behaviour is typical of ZnO nanoparticles, which are known for broad spectrum UV blocking. Upon conjugation with BSA, new notable absorption peak appears at lower wavelength (218 nm) demonstrating the formation of ZnO-protein conjugate (Fig. 4). Researchers in the past have reported that during nanoparticles-BSA conjugation, the protein undergoes substantial conformational changes at both secondary and tertiary structure levels. These changes are related with the pH of the reaction medium, which determines the extent of blue shift in UV region.

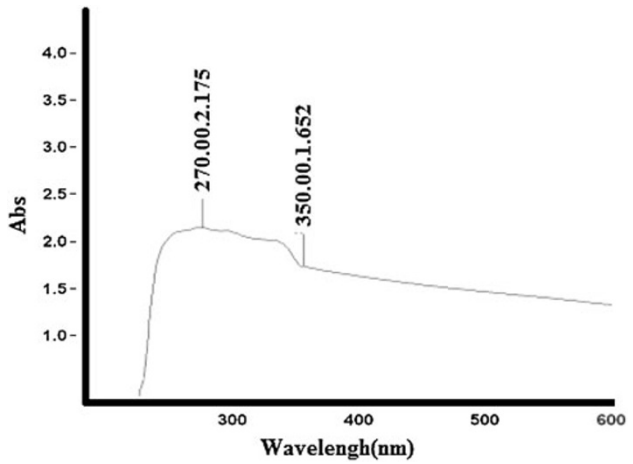

Fig. 3 UV–Vis spectra of ZnO nanoparticle

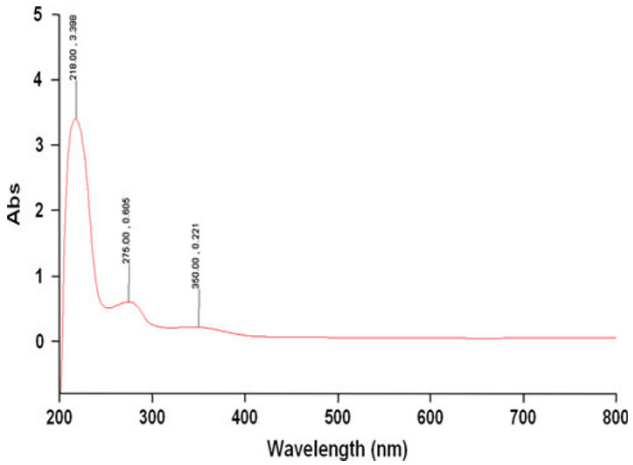

Fig. 4 UV–Vis spectra of ZnO-BSA conjugate

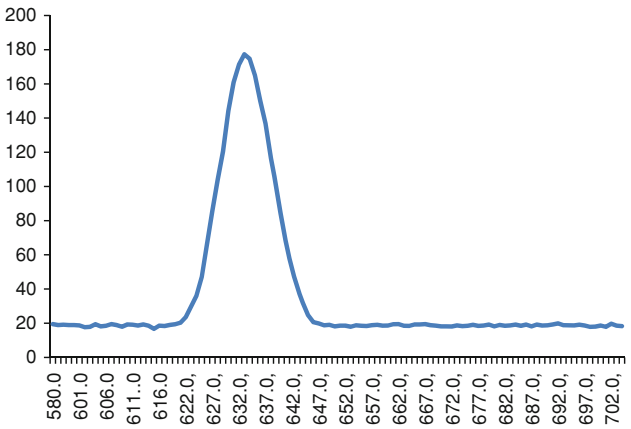

Fig. 5 Photoluminescence intensity of ZnO-BSA conjugates

The fluorescence spectra of the ZnO-BSA solutions indicate that the fluorescence spectra changed obviously when ZnO conjugated with BSA. The photoluminescence peak position from 640 nm for pure ZnO emission to 633 nm for ZnO-BSA was accompanied with the decrease in the fluorescence intensity after the addition of BSA (Fig. 5).

Conclusions and future scope

It has been demonstrated that ZnO nanoparticles can be conjugated with BSA. Further studies are required to conclude the mechanism of conjugation, optimization of parameters, fluorescence studies and structural investigations of the prepared conjugate. ZnO nanoparticles or similar kind of fluorescent nanostructures can be conjugated with specific biomarkers for the identification of breast cancer cells.

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